

Old Claim No.	New Claim No.	Status
88	178	allowed
89	179	allowed
90	180	allowed
94	182	allowed
117	184	
118	185	allowed
119	186	allowed
120	187	allowed
121	188	
122	189	
123	190	
124	191	allowed
126	192	
127	195	
128	196	
129	197	
130	198	
131	199	
132	200	
133	201	
134	202	
135	203	allowed
137	193	allowed
138	204	allowed
139	205	allowed
140	206	allowed
142	207	allowed
143	208	
144	209	

Old Claim No.	New Claim No.	Status
145	210	
146	211	
147	212	
148	213	
149	214	
150	215	allowed
151	216	allowed
152	221	allowed
153	225	
154	217	allowed
155	218	allowed
156	219	allowed
157	220	allowed
158	cancel	cancel
159	229	allowed
160	230	allowed
165	231	allowed
166	232	allowed
167	233	allowed
168	234	allowed
169	222	allowed
170	223	allowed
171	224	allowed
172	226	
173	227	
174	228	
175	181	allowed
176	183	allowed
177	194	allowed

Please add the following claims:

--178. A purified and isolated nucleic acid sequence for bacterial expression of a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 and an amino-terminal methionine residue.

179. An expression vector for bacterial expression of a glial cell line-derived neurotrophic factor polypeptide comprising a nucleic acid sequence according to Claim 178.

180. An isolated host cell for bacterial expression of a glial cell line-derived neurotrophic factor polypeptide comprising the nucleic acid of Claim 178.

181. A host cell comprising *E. coli* transformed or transfected with a nucleic acid sequence according to Claim 178.

182. A method for the bacterial production of glial cell line-derived neurotrophic factor polypeptide comprising the steps of:

- (a) culturing a bacterial host cell containing a nucleic acid sequence according to Claim 178;
- (b) maintaining said host cell under conditions allowing the expression of glial cell line-derived neurotrophic factor polypeptide by said host cell; and
- (c) optionally, isolating the glial cell line-derived neurotrophic factor polypeptide expressed by said host cell.

183. A method of production according to Claim 182 wherein said host cell is *E. coli*.

184. A purified and isolated DNA sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence:

- (a) comprises nucleotides 304 through 705 of SEQ ID NO:3 or nucleotides 105 through 506 of SEQ ID NO:5; or
- (b) encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; or
- (c) hybridizes to an oligonucleotide probe fully complementary to a nucleic acid sequence encoding the amino acid sequence of SEQ. ID. NO:6 under conditions comprising hybridizing said sequences in 6X SSPE and 0.1% SDS at 50°C, followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons; or

(d) hybridizes to an oligonucleotide probe fully complementary to a nucleic acid sequence encoding amino acids 2 to 86 of SEQ ID NO:4 under conditions comprising hybridizing said sequences in 6X SSPE, 0.1% SDS and 30% formamide at 42°C, followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

185. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, said nucleic acid sequence comprising nucleotides 105 through 506 of SEQ ID NO:5, wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

186. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:6, wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

187. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

188. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, comprising a sequence which hybridizes to an oligonucleotide probe fully complementary to a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:6 under conditions comprising hybridizing said sequences in 6X SSPE and 0.1% SDS at 50°C, followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C, or to an oligonucleotide probe encoding amino acids 2 to 86 of SEQ ID NO:4 under conditions comprising hybridizing said sequences in 6X SSPE, 0.1% SDS and 30% formamide at 42°C, followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C, and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

189. A purified and isolated nucleic acid sequence according to claim 184, 185, 186, 187 or 188 further comprising a codon encoding an amino-terminal methionine residue when said polypeptide is recombinantly produced by a bacterial expression system.

190. A purified and isolated nucleic acid sequence comprising a sequence complementary to a nucleic acid sequence according to claim 184, 185, 186, 187 or 188.

191. A purified and isolated nucleic acid sequence according to claim 185 or 186 further comprising nucleotides encoding a pre-pro amino acid sequence as set forth in SEQ ID NO:26 amino acid residues 10 through 86.

192. A vector comprising a nucleic acid sequence according to claim 184, 185, 186, 187 or 188.

193. A vector comprising a purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence of SEQ ID NO:6.

194. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a host cell transformed or transfected with a vector according to claim 193 under conditions suitable for the expression of glial cell line-derived neurotrophic factor polypeptide; and optionally
- (b) isolating said glial cell line-derived neurotrophic factor polypeptide expressed by said host cell.

195. An isolated host cell comprising a nucleic acid of claim 184, 185, 186, 187 or 188.

196. A host cell according to claim 195 wherein said host cell is an animal cell.

197. A host cell according to claim 195 wherein said host cell is a COS cell.

198. A host cell according to claim 195 wherein said host cell is a microorganism.

199. A host cell according to claim 195 wherein said host cell is a bacterial cell.

200. A host cell according to claim 195 wherein said host cell is an *E. coli* cell.

201. A host cell according to claim 195 wherein said cell is transformed or transfected *ex vivo* and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

202. A host cell according to claim 195 wherein said cell is suitable for human implantation and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

203. An isolated host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, said nucleic acid sequence operatively linked to a non-native promoter, wherein said nucleic acid sequence:

- (a) comprises nucleotides 105 through 506 of SEQ ID NO:5; or
- (b) encodes a polypeptide comprising an amino acid sequence of SEQ ID NO:6.

204. An isolated host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence of SEQ ID NO:6.

205. A host cell according to claim 204 wherein said host cell is a microorganism.

206. A host cell according to claim 204 wherein said host cell is an animal cell.

207. A host cell according to claim 204 wherein said cell is transformed or transfected *ex vivo* and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

208. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a host cell according to claim 195 under conditions suitable for the expression of glial cell line-derived neurotrophic factor polypeptide; and optionally
- (b) isolating said glial cell line-derived neurotrophic factor polypeptide therefrom.

209. A method according to claim 208 wherein said host cell is an animal cell.

210. A method according to claim 209 wherein said host cell is a COS cell.

211. A method according to claim 208 wherein said host cell is a microorganism.

212. A method according to claim 211 wherein said host cell is *E. coli*.

213. A method according to claim 208 further comprising the step of refolding said glial cell line-derived neurotrophic factor polypeptide.

214. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a host cell transformed or transfected with a vector according to claim 192 under conditions suitable for the expression of glial cell line-derived neurotrophic factor polypeptide; and optionally
- (b) isolating said glial cell line-derived neurotrophic factor polypeptide expressed by said host cell.

215. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is

operatively linked to a non-native promoter, and wherein said nucleic acid sequence is selected from the group consisting of:

- (i) nucleotides 304 through 705 of SEQ ID NO:3 or nucleotides 105 through 506 of SEQ ID NO:5; or
- (ii) nucleotides encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; and

(b) isolating said expressed polypeptide in a substantially purified form from said host cell culture.

216. A method according to claim 215 wherein said nucleic acid further comprises a codon encoding an amino-terminal methionine residue when said polypeptide is recombinantly produced by a bacterial expression system.

217. A method according to claim 215 wherein said host cell is an animal cell or microorganism.

218. A method according to claim 215 further comprising refolding expressed glial cell line-derived neurotrophic factor polypeptide to form a disulfide-bonded dimer.

219. A method according to claim 215 wherein said glial cell line-derived neurotrophic factor polypeptide is expressed by a bacterial host cell and is refolded to form a disulfide-bonded dimer.

220. A method according to claim 215 wherein the expressed glial cell line-derived neurotrophic factor polypeptide is secreted by said host cell.

221. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence

encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; and

(b) isolating said expressed polypeptide in a substantially purified form from said host cell culture,

wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

222. A method according to claim 221 wherein said host cell is an animal cell or microorganism.

223. A method according to claim 221 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

224. A method according to claim 221 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.

225. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

(a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence encodes a polypeptide having the capability to promote dopamine uptake in dopaminergic neurons and

(i) hybridizes to an oligonucleotide probe fully complementary to a nucleic acid sequence encoding the amino acid sequence of SEQ. ID. NO:6 under conditions comprising hybridizing said sequences in 6X SSPE and 0.1% SDS at 50°C, followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C; or

(ii) hybridizes to an oligonucleotide probe fully complementary to a nucleic acid sequence encoding amino acids 2 to 86 of SEQ ID NO:4 under conditions comprising hybridizing said sequences in 6X SSPE, 0.1% SDS and 30% formamide at 42°C,

followed by washing in 2X SSPE and 0.1% SDS at room temperature and twice in 0.1x SSPE, 0.1% SDS preheated to 50°C; and

(b) isolating said expressed polypeptide in a substantially purified form from said host cell culture.

226. A method according to claim 225 wherein said host cell is an animal cell or microorganism.

227. A method according to claim 225 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

228. A method according to claim 225 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.

158. (Cancel) A purified and isolated nucleic acid molecule according to claim 184 which encodes glial cell line-derived neurotrophic factor having a molecular weight of about 31-42 kD on non-reducing SDS-PAGE, a molecular weight of about 20-23 kD on reducing SDS-PAGE, and which promotes dopamine uptake in dopaminergic neurons at a concentration of approximately 60 pg/ml.

229. An isolated host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence comprises nucleotides 105 through 506 of SEQ ID NO:5, operatively linked to a non-native promoter; and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

230. An isolated host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

231. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence comprises nucleotides 105 through 506 of SEQ ID NO:5; and
- (b) isolating said expressed polypeptide in a substantially purified form from said host cell culture,

wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

232. A method according to claim 231 wherein said host cell is an animal cell or microorganism.

233. A method according to claim 231 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

234. A method according to claim 231 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.--

Please cancel claims 88-90, 94, 117-135, 137-140, 142-160, 165-177, without prejudice, to be replaced by the newly added claims.

#### **REMARKS**

Claims 88-90, 94, 118-120, 124, 135, 137-140, 142, 150, 152, 154-157, 160, 165-171, and 175-177 were previously allowed. The claims have been renumbered and reordered at the Examiner's request to facilitate completion of the reopened examination. The claims reflect the amendments made up to and through August 15, 2000.